diagenode
A Hologic Company

PROTOCOL

Transposome assembly using Diagenode Tagmentase

DESCRIPTION

Diagenode Tagmentase is a hyperactive Tn5 transposase. Its ability to cut DNA and insert sequencing adapters in one step makes it the perfect companion for Next-Generation Sequencing experiments using powerful technologies such as ATAC-seq or ChIPmentation.

For flexibility of use, the protein is not pre-loaded with sequencing adapters and should be loaded with appropriate oligonucleotides prior to use. Oligonucleotides should contain 19-mer Tn5 mosaic ends (underlined) recognized by the transposase and the sequences (bold) allowing the PCR amplification e.g., with Illumina-compatible barcoded i7/i5 primers. These sequences have to be adapted to a particular experimental design and take into account the sequencing platform requirements.

Mosaic end_reverse: [PHO]CTGTCTCTTATACACATCT

Mosaic end_Adapter A: **NNNNNNNNNNNNNNN**AGATGTGTATAAGAGACAG

Mosaic end_Adapter B: **NNNNNNNNNNNNNNN**AGATGTGTATAAGAGACAG

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1. Order the oligos that you would like to use to load the tagmentase. You will need 3 oligos that we can call A, B and Rev. They should be lyophilized.
2. Prepare the following Annealing Buffer: 40mM Tris-HCl (pH8.0), 50mM NaCl.
3. Resuspend the oligos in Annealing Buffer to stock concentration of 100 µM.
4. In a PCR tube, mix 10 µl of oligo Rev with 10 µl of oligo A.
5. In a separate PCR tube, mix 10 µl of oligo Rev with 10 µl of oligo B.
6. Vortex and place PCR tubes in a thermocycler.
7. Run the following programme:

Temperature	Time
95°C	5 minutes
Cool to 65°C	-0.1°C/second
65°C	5 minutes
Cool to 4°C	-0.1°C/second

Note: Annealed linker oligos can be stored at -20°C.
You can therefore prepare a bigger volume and freeze it.

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8. In a PCR tube, mix 5 µl of the annealed oligo A/oligo Rev with 5 µl of the annealed oligo B/oligo Rev.
9. Add 10 µl of Diagenode Tagmentase (Cat. No. C01070010).
10. Pipet gently and incubate at 23°C for 30 minutes in a thermocycler.

    Caution: *Do not exceed 60 minutes incubation time, or the Tagmentase will lose activity.*

11. If not for immediate use, add 10 µl of glycerol and store at -20°C.

    Note: If dilution of the transposome is needed we recommend using the Tagmentase Dilution Buffer which is available separately (Cat. No. C01070011). This buffer contains 50% glycerol.

REFERENCE

Picelli S, Björklund AK, Reinius B, Sagasser S, Winberg G, Sandberg R. Tn5 transposase and tagmentation procedures for massively scaled sequencing projects. Genome Res. 2014;24(12):2033–2040. doi:10.1101/gr.177881.114

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